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Some Observations on the Reproductive Anatomy of *Isoetes andicola*

ERIC KARRFALT
10261 Fry Rd., McKean, PA 16426

ABSTRACT.—The gametophyte generation of *Isoetes andicola* was investigated anatomically and using microspectrophotometry. Microsporangia, which sometimes additionally contained a few abortive megaspores, produced numerous microspores that developed apparently functional male gametophytes and swimming spermatozoids. In contrast, the female gametophytes produced from functional megaspores were found to contain archegonia lacking neck canals and that mostly did not develop functional embryos. Instead, the megagametophytes were found to contain embryos deeply embedded within somatic tissue. The nuclei of these embryos were about twice the size and contained about twice as much DNA as of those in adjacent gametophytic cells. Such embryos, which were not associated with archegonia, are interpreted to have arisen via some form of apogamy.

Plants that are now referable to *Isoetes andicola* (Amstutz) L.D. Gómez were originally described as two separate species, *Stylites andicola* E. Amstutz and *S. gemmifera* Rauh. Although Gómez (1980) had maintained the latter taxon as a variety of *I. andicola,* this distinction is dubious (Karrfalt and Hunter, 1980; Karrfalt, 1984), and although Gómez (1980) did not provide any new evidence to support the combination of *Stylites* with *Isoetes,* Karrfalt (1984) showed that the elongate, monopolar stem characterizing the genus *Stylites* is secondarily derived during the ontogeny of each young *Stylites* plant and that these plants begin life with bipolar corms that are morphologically indistinguishable from those of any other species of *Isoetes*. As an adjunct to this study of stem development, plants of *I. andicola* maintained in cultivation yielded a large number of gametophytes that subsequently produced correspondingly large numbers of new sporophytes. These gametophytes and sporophytes provided an unexpected opportunity to study some aspects of the reproductive anatomy of this taxon. These anatomical observations seem to suggest that apomixis exists in this species.

METHODS AND MATERIALS

The material used in this study was collected and maintained as reported previously (Karrfalt and Hunter, 1980; Karrfalt, 1984). The observations reported here were made over a period of eight months beginning when the megaspore walls first began to open and continued until the food reserves of the gametophytes (as seen in sectioned material) was essentially exhausted. Because the original intent of growing the gametophytes was simply to obtain additional sporophytes, both megaspores and microspores were sown together. The possibility of conducting the present study only became apparent after

the spores had germinated. Inferences about the developmental fate of the archegonia and hence their involvement or not in sexual reproduction were possible because the serial sections were all complete and the series of sections uninterrupted. All cells of all archegonia could be accounted for readily.

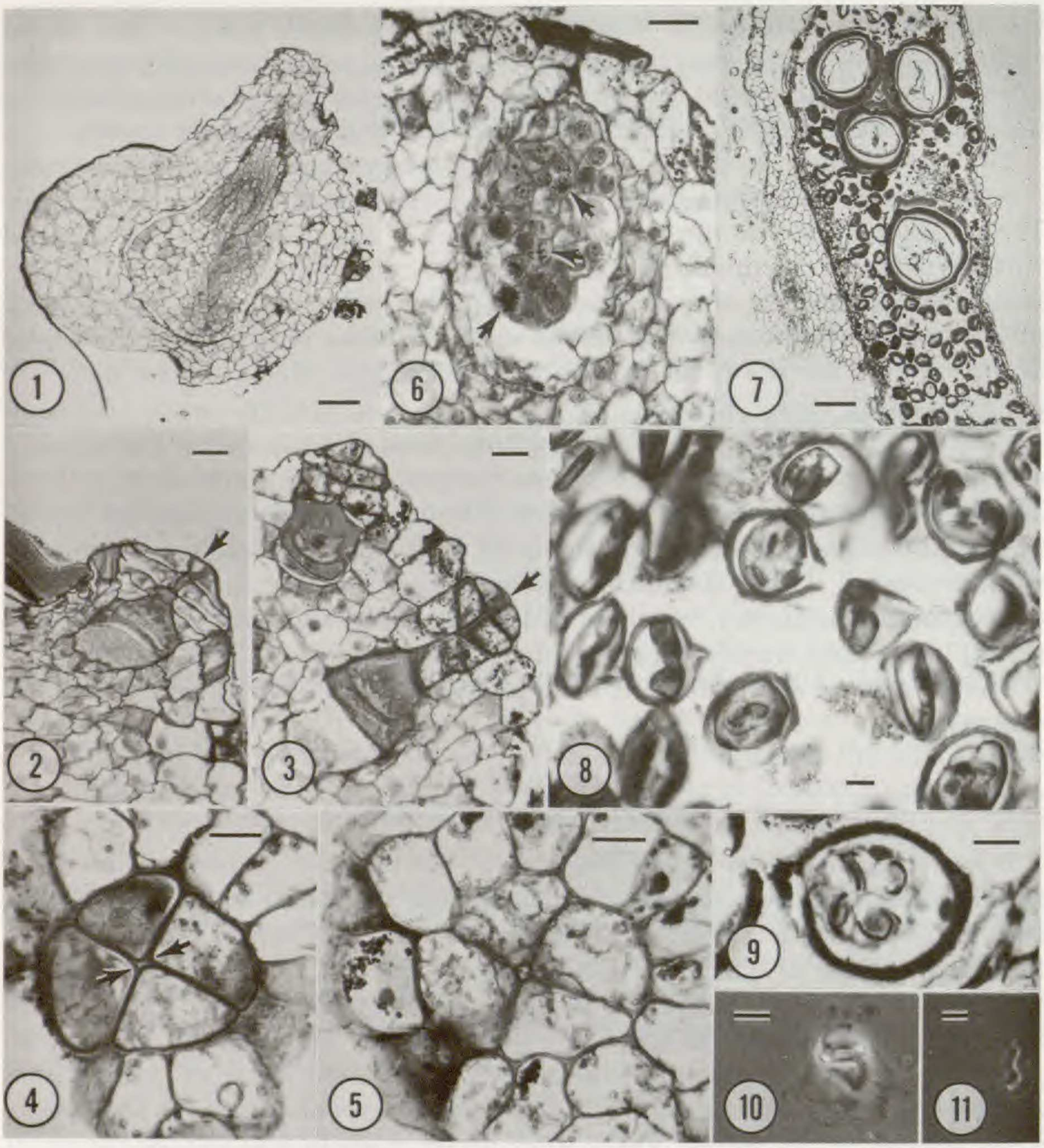
The material used in this study was processed by standard paraffin methods. A total of 378 archegonia in 62 gametophytes was studied in serial sections. The megagametophytes mostly were sectioned at right angles to their exposed surfaces so as to cut the maximum number of archegonia in longitudinal section. This orientation was obtained readily by embedding the megagametophytes under a dissecting microscope so that a plane tangential to the center of the exposed surface of the gametophyte was perpendicular to the bottom of the embedding vessel. Sections were then cut parallel to the lower surface of the paraffin block. In a few cases, sections were cut parallel to the exposed surface of the gametophyte so as to view the archegonia in serial cross sections. The megagametophytes were sectioned at 6 μm, except those used for microspectrophotometry, which were sectioned at 20 μm. The microgametophytes were processed within the dead microsporangia and sectioned at 4 μm. The microspectrophotometry was done by the two wavelength method of Ornstein (1952) using light wavelengths of 550 nm and 495 nm.

The swimming spermatozoids were photographed under phase contrast microscopy.

Results and discussion

When the megaspore suture first opens, the exposed surface of the megagametophyte does not protrude at all beyond the spore wall. With further growth, however, the megagametophyte enlarges considerably and eventually reaches a volume of which only half or less can be contained within the spore wall. Subsequent to the opening of the suture, new archegonia are produced continually throughout the life of the gametophyte. The largest number of archegonia on a single gametophyte was 23. A detailed study of gametophyte development was not attempted, but the process seems to be the same as in other species of the genus. There is an initial stage of free-nuclear divisions followed by a gradual cellularization beginning under the trilete scar portion of the megaspore wall. Eventually, cellularization is essentially complete, although occasional binucleate cells may be found.

After sporelings began to emerge from the gametophytes in the cultures, a search was begun to identify earlier stages of sporophyte development. Samples consisting of ten megagametophytes were selected from time to time and processed so as to examine for embryos within a few days after removal from cultures. Each sample was selected to include a more or less full spectrum of of gametophyte sizes. Initially, the only embryos found were in an advanced state of development, with well-defined primordial organs. These were always located deep within the tissue of the gametophyte and not associated with an archegonium (Fig. 1). In every megagametophyte containing an embryo not associated with an archegonium, all of the archegonia were still intact, com-



FIGS. 1–11. *Isoetes andicola*. 1) Section of a megagametophyte containing a sporophyte not associated with an archegonium. 2, 3) Longitudinal sections of mature archegonia; arrows mark spurious neck canals. 4) Cross-section of an archegonial neck through the second tier from the distal end. 5) Cross-section of an archegonial neck through the third tier from the distal end. 6) Longitudinal section of an archegonium lacking the outer two tiers of neck cells and containing vigorously growing embryo; arrows mark mitotic figures. 7) Longitudinal section of a microsporangium containing some small abortive megaspores. 8) Variously sectioned microspores and microgametophytes. 9) Longitudinal section of a mature microgametophyte containing four coiled spermatozoids, remnants of jacket cells, and a prothallial cell (at the righthand end). 10) Spermatozoid during the slowly swimming stage. 11) Spermatozoid during the rapidly swimming stage. Scale bars: 1, 7 = 100 μm; 2–6, 11 = 25 μm; 8–10 = 10 μm.

plete with an undivided egg cell in the venter. The nuclei of the embryo cells were about twice the diameter of those in the surrounding gametophyte tissue, suggesting that in spite of the lack of participation of an egg cell, the origins of these embryos nonetheless involved an increase in ploidy level. Microspectrophotometric measurements of gametophytic nuclei and sporophytic nuclei confirmed that this was the case. The mean relative amount of DNA for the gametophytic nuclei (9.75 ± 1.11, n = 9) was about half that of the sporophytic nuclei (18.4 ± 1.79, n = 11), and that of the maximum measurement for gametophytic nucleus (13.1, presumably 2c) was very similar to the minimum measurement from among the sporophytic nuclei (13.7; also presumably 2c, where the quantity of DNA in a haploid nucleus prior to DNA synthesis = c, the quantity of DNA of the completion of DNA synthesis and in a diploid nucleus prior to DNA replication = 2c, and at the completion of DNA synthesis = 4c).

The mature archegonia had necks consisting of four tiers of neck cells. At first, it appeared that a canal ran through the length of the neck from the venter to the exterior, but closer examination showed that this was not the case. Some archegonia appeared to show a canal in longitudinal section (Figs. 2, 3, arrow), but others showed no "canal" in the outer two tiers of neck cells anywhere in the complete series of relatively thin sections (Fig. 3, left archegonium). Comparisons of longitudinal sections of serial cross sections (Figs 4, 5) revealed that there were no neck canal cells and no neck canal in the outer two tiers of the neck, but that the ventral canal cell extended through the inner two tiers of neck cells. The superficial appearance of a canal through the outer two tiers of some of the necks in longitudinal sections resulted when the plane of section was such as indicated by the opposed arrows in Fig. 4. The "canal" through the outer two tiers in these cases was either simply a face view of the wall between the longitudinal files of neck cells on opposite sides of the neck (Fig. 3, arrow) or some combination of that wall and the lumen of one of the cells sharing that wall (Fig. 2, arrow). Rauh and Falk (1959) refer to an unspecified number of neck canal cells passing throught the outer two tiers of the archegonial neck, but their micrograph of such an archegonium (Rauh and Falk, 1959, Abb. 34 III) is so similar to Fig. 2 that I suspect their identification of neck cells may have been mistaken.

Of the 62 megagametophytes that were studied in serial sections, 3 possessed a few archegonia that lacked the outermost tier of neck cells. These archegonia were obviously moribund, with most of their remaining cells apparently suberized. In the entire study, only one archegonium was found that was missing the outer two tiers of neck cells, i.e., its neck canal was open to the exterior. The venter of this archegonium contained a vigorous embryo (Fig. 6, note three mitotic figures at arrows). Attempts to manually break off the outer two tiers of neck cells to facilitate fertilization resulted in no embryo formation. Near the end of this study, embryos began to occur within archegonia fairly frequently. Twenty such embryos were found, but nine of these appeared to be in poor condition or were dead. One intact archegonium con-

tained a living embryo with each of its four cells in late anaphase and hence presumably in good health.

Whether or not gametic union ever occurs in this plant, apparently normal male gametophytes and spermatozoids are produced. Although some microsporangia contain small abortive megaspores in addition to microsproes (Fig. 7) and all microsporangia examined contained substantial numbers of abortive microspores with collapsed protoplasts (Fig. 8, to the right and left of center), numerous mature and immature male gametophytes were seen as well (Fig. 8, center). The mature male gametophytes contain four large coiled spermatozoids surrounded by the remnants of jacket cells and a single prothallial cell (Fig. 9), as in other species of *Isoetes*. The spermatozoids swim vigorously and in this respect at least appear perfectly normal. A wet mount of some contents of a microsporangium would usually include some swimming spermatozoids. If not, slight pressure on the cover slip with a dissecting neeedle would cause one or more mature antheridia to dehisce. Upon emergence, each spermatozoid uncoils slightly (Fig. 10) and begins to rotate and swim slowly. The numerous flagella then begin to beat more rapidly and the spermatozoids uncoil further (Fig. 11). They progress so rapidly through the water that it can be difficult to keep them in view. The rapid swimming persists for about one minute, after which the spermatozoids slow down and recoil to essentially their initial shape.

The embryos that occur deep within the gametophytic tissue and are not associated with archegonia are apparently apogamous. The anatomical observations definitely preclude the participation of either egg or spermatozoid in the formation of these deep-seated embryos. The present observations do not reveal specifically how these embryos are initiated, but the microspectrophotometric results show unequivocally that the ploidy level of these embyos is double that of the surrounding gametophytic tissue. Possibly the deep-seated embryos are initiated by endoreduplication in single vegetative gametophytic cells or possibly the nuclei of binucleate cells fuse. Occasional binucleate cells have been seen in *I. taiwanensis* De Vol (Huang and Chiang, 1986) and are not unique to *I. andicola*. In *I. andicola*, however, they may have acquired a novel function, that of embryo formation.

Those embryos in the venters of archegonia with intact unopened necks apparently are also apogamous, as were almost all of the embryos seen in this study. The only embryo observed that might have resulted from gametic union is that shown in Fig. 6. If this was a sexually produced embryo, then presumably the outer two tiers of the archegonial neck were lost prior to fertilization. In the absence of any evidence of a neck canal through the outer two tiers of neck cells in any of the archegonia, it would seem that a sperm could only reach the egg following the loss of these outer tiers of neck cells, as occurs in *Psilotum* and *Stromatopteris* (Bierhorst, 1968). The extreme rarity with which archegonia lacking the outer two tiers of neck cells were observed would seem to indicate that loss of these cells is not a normal developmental event. The possibility cannot be excluded, however, that under field conditions or in material from other localities the archegonia might develop differently.

The usual persistence of the outer two tiers of neck cells and the absence of a neck canal through this part of the neck apparently ensures that if fertilization ever occurs it is a rare event. In any case, the embryos that are not associated with archegonia and those contained within archegonia with intact unopened necks are most likely apomictic. Regardless of their origin, the sporophytes produced by megagametophytes probably play a relatively minor role in the maintenance of populations of this species. Gómez (1980) found that vegetatively produced plants of *I. storkii* T.C. Palmer are much more likely to grow into adult plants and in less time than sexually produced plants, because the latter are very much smaller and more fragile than the former. The abundant robust gemmae produced by *I. andicola* would likewise seem to have a competetive advantage over the minute sporophytes produced by gametophytes in the crowded conditions (Karrfalt and Hunter, 1980) in which these plants grow.

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LITERATURE CITED

BIERHORST, D. W. 1968. On the Stromatopteridaceae (fam. nov.) and the Psilotaceae. Phytomorphology 18:232–268.

GÓMEZ, L. D. 1980. Vegetative reproduction in a Central American *Isoetes* (Isoetaceae), its morphological, systematic, and taxonomical significance. Brenesia 18:1–14.

HUANG, S.-F., and S.-H. T. CHIANG. 1986. The development of the female gametophyte in *Isoetes taiwanensis* De Vol. Taiwania 31:15–32.

KARRFALT, E. 1984. The origin and early development of the root-producing meristem of *Isoetes andicola* L.D. Gómez. Bot. Gaz. (Crawfordsville) 145:372–377.

KARRFALT, E., and M. HUNTER. 1980. Notes on the natural history of *Stylites gemmifera*. Amer. Fern J. 70:69–72.

ORNSTEIN, L. 1952. The distributional error in microspectrophotometry. Lab. Invest. 1:250–262.

RAUH, W., and H. FALK. 1959. *Stylites* E. Amstutz, eine neue Isoetacee aus den Hochanden Perus. I. Teil: Morphologie, anatomie, und Entwicklungsgeschichte der Vegetationsorgane. Sitzungsber. Heidelberger Akad. Wiss. Math. Naturwiss. Kl. 1959:1–83.